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Locations of Genetic Markers on the Physical Map of the Chromosome of *Neisseria gonorrhoeae* FA1090

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To increase the utility of the previously constructed physical map of the chromosome of *Neisseria gonorrhoeae* FA1090, 28 additional genetic markers were localized on the map. Cloned gonococcal genes were used to probe Southern blots of restriction enzyme-digested DNA separated on pulsed-field gels, thus identifying the fragment in each of several digests to which the probe hybridized and the map location of each gene. The addition of the new markers brings the total number of mapped loci for this strain to 68; the locations of all of those markers on the updated map are shown.

Neisseria gonorrhoeae, the gram-negative diplococcus that is the causative agent of the sexually transmitted disease gonorrhea, has been the focus of much effort devoted to understanding the molecular pathogenesis of gonococcal infection. These studies have resulted in the identification of numerous genes and gene families and in the recognition that there are novel aspects to gene organization and regulation in this species. Because genetic maps have been such powerful tools for the study of other, more extensively characterized bacteria, there has been interest in developing a detailed map of the gonococcal chromosome. In the gonococcus, the exchange of chromosomal markers is limited to transformation; the small sizes of DNA segments transferred via this process make the construction of a map by transformation an unrealistic objective (4, 43). However, as with a number of other species, physical mapping techniques have provided an effective means for developing a map of the gonococcal chromosome.

We constructed a macrorestriction map of the circular 2.2-megabase chromosome of strain FA1090, ordering the fragments produced by digestion with the enzymes *Nhe*I and *Spe*I, which have rare recognition sequences. The resolution of the map was increased by partial mapping of the larger, *Pac*I and *Bgl*II fragments (11). Bihlmaier et al. constructed *Nhe*I and *Spe*I maps of the chromosome of strain MS11 (3). These two strains are well characterized, and each has been used in studies on genetic regulation and pathogenesis, including experimental infection of human volunteers (6, 40, 41). The overall organization of the chromosome is nearly identical in the two strains, with the locations of most of the mapped genetic markers being the same (within the limits of resolution of the maps).

In the time since the construction of macrorestriction maps, a number of additional gonococcal genes have been cloned and characterized. As part of a continuing effort to make the physical map a more valuable resource for genetic studies of the gonococcus, we have determined the locations on the FA1090 map of a collection of 28 new genetic markers that were provided to us by other investigators.

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MATERIALS AND METHODS

Bacterial strains. *N. gonorrhoeae* FA1090 is a serum-resistant, streptomycin-resistant, proline-requiring strain isolated in 1983 from a patient with disseminated gonococcal infection (7, 8). Gonococci were grown in GC broth or on GC agar (Difco Laboratories) with the supplements of Kellogg et al. (19) at 37°C in a 5% CO_2 atmosphere. *Escherichia coli* strains were grown on LB agar (31).

Pulsed-field electrophoresis. The methods used for the preparation of gonococcal DNA in agarose blocks, digestion of DNA with restriction enzymes, and pulsed-field electrophoresis in a contour-clamped homogeneous electric field apparatus were as described previously (11). Samples were run on 1% agarose gels containing either individual wells or one well extending the width of the gel. Different pulse times were used to resolve particular restriction fragments.

DNA transfer and hybridization. The procedures used for nicking DNA with UV light, transferring DNA fragments to supported nitrocellulose filters (Schleicher & Schuell, Inc.), and cross-linking the DNA to the filters were as described previously (11). Filters from gels with one continuous sample well were cut into narrow strips, which were hybridized with different probes. Probe-hybridizing fragments were identified unambiguously by overprobing the strips with radiolabeled FA1090 chromosomal DNA to mark all fragments (11). DNA fragments were labeled with ^{32}P by random priming (Pharmacia); hybridization and washing procedures were as described previously (11).

RESULTS AND DISCUSSION

We probed Southern blots of FA1090 DNA digested with each of four mapping enzymes by using cloned genes or portions of genes that we obtained from other investigators. Digestion of FA1090 DNA with *Spe*I, *Nhe*I, *Pac*I, and *Bgl*II generated 17, 16, 21, and 30 fragments, respectively. All of the *Spe*I and *Nhe*I fragments have been mapped, as have the 9 largest *Bgl*II fragments and the 10 largest *Pac*I fragments (11). Table 1 lists the gene probes and the restriction fragments to

TABLE 1. Cloned gene probes and fragments to which they hybridized

Clone	Gene symbol (description or phenotype)	Hybridizing fragment			
		*Nhe*I	*Spe*I	*Bgl*II	*Pac*I
—[a]	*adk* (adenylate kinase)	N2	S3	B2	P7
pKS4.21	*aniA* (anaerobically induced protein Pan1)	N3	S4	<B9[b]	P6
pNM1	*asd* (aspartate semialdehyde dehydrogenase [*N. meningitidis*])	N4	S6	ND[c]	ND
pFL35	*carB* (carbamoylphosphate synthetase)	N1	S9	B1 +[d] <B8	P3
pUNCH325	*frpB* (70-kDa iron-repressible outer membrane protein)	N10	S6	ND	ND
pUNCH601	*fur* (ferric uptake regulator)	N9	S8	B5	P5
pGC.P11	*gyrA* (DNA gyrase subunit)	N2	S10	B4	P1
pGC.P13	*himA* (integration host factor subunit)	N1	S14	B2	P7
pGC.P14	*himD* (integration host factor subunit)	N2	S13	ND	ND
—	*hsp-63* (63-kDa stress protein GSP63)	N10	S6	B1	P2
pUNCH122	*lbpA* (lactoferrin-binding protein)	N1	S5	B3	<P8
pGC.P17	*lepA* (GTP membrane-binding protein [function unknown])	N2	S3	B2	P7
pSP931[e]	*lpd* (lipoamide dehydrogenase)	N3	S2	<B8	P1
—	*mtrR* (increased resistance to antibiotics and hydrophobic compounds)	<N12	S4	B6	P4
pG4	*parC* (topoisomerase activity)	N3	S4	<B10	P6
p12/7/1[f]	*pilD* (prepilin leader peptidase)	N8	S1	<B8	<P10
p12/7/1[f]	*pilF* (pilin assembly)	N8	S1	<B8	<P10
p2a2	*pilT* (pilus-associated twitching motility and colony morphology)	N4	S11	ND	ND
pNG200	*regF* (regulation of *pilE* transcription)	N1	S6	B1	P3
p14B8	*rrn* (rRNA [*Bacillus subtilis*])	N4	S11	ND	P2
		N8	S1	B5	P5
		N11	S4	ND	P4
		N3	S12	ND	P6
pSP931[e]	*sucA* α-ketoglutarate dehydrogenase [decarboxylase])	N3	S2	<B8	P1
pSP961	*tufA* (elongation factor [EF-Tu])	N4	S8	ND	P2
pJKD966	*uvrA* (excision nuclease [DNA repair])	N3	S12	<B8	P6
pJKD960	*uvrB* (excision nuclease [DNA repair])	N2	S13	B4	ND
pGC.P16	*uvrC* (excision nuclease [DNA repair])	N2	S13	B4	P1

[a] —, no clone designation provided.
[b] The less-than symbol indicates that the hybridizing fragment was smaller than the fragment indicated, but its identity was not determined.
[c] ND, not done.
[d] Clone pFL35 contains a *Bgl*II site and is therefore a linking clone for two *Bgl*II fragments.
[e] The *lpd* and *sucA* genes were cloned on a single cosmid; gene-specific probes were generated by the PCR.
[f] The *pilD* and *pilF* genes were cloned on a single plasmid; gene-specific probes were generated by PCR.

which the probes hybridized. The results of the Southern blotting experiments allowed us to localize each gene to a region of the chromosome consisting of the overlap between fragments from different digests, as indicated on the map in Fig. 1. The results for all of the gene probes were consistent with the previously constructed macrorestriction map (11).

On a physical map of this type, it is possible to localize a gene to a particular region of overlap between restriction fragments from different digests but not to determine the relative order of multiple genes in that region. Because of this uncertainty about the relative order and locations of markers within each region of overlap, we believe it is premature to attempt to devise a system of map coordinates and assign a unique map location for each gene. Also, such coordinate systems often use the origin of replication as the starting point for numbering. By analogy with other gram-negative bacteria, it is likely that the origin of replication of the gonococcal chromosome is located near the *gyrB* gene at 12 o'clock on the map (27). However, experimental verification of this prediction is necessary before the origin of replication can be used as a landmark on the physical map and before a permanent system of map coordinates can be developed.

The locations of previously mapped genetic loci (11) are also indicated on the map in Fig. 1, and Table 2 lists all of the genetic loci that we have placed on the map to date, as well as the names of the investigators who provided the probes or the reference in which each probe is described. To make it easier to find the genetic loci listed in Table 2, we have divided the circular map into 10 approximately equal arbitrary zones, designated A to J. Each of these zones represents one or more regions of overlap between the *Nhe*I and *Spe*I fragments, and the zone into which each marker falls is indicated in Table 2. The division of the map into these zones is not intended as a precursor to a permanent system of coordinates but is only an interim aid for locating markers on the map.

Our original construction of the map involved the use of anonymous clones of FA1090 DNA to identify fragment overlaps and to provide additional markers for different locations on the map (11). Because the specific genes contained on these clones have not been identified, we have not included them on this representation of the map.

Even with the limitations imposed by the relatively low resolution of the current map, it is becoming possible to obtain useful information from it. The physical relationships between genes that affect similar pathways or phenotypes can be determined, perhaps shedding light on possible regulatory mechanisms. For example, loci affecting the synthesis and assembly of pili (*pilC*, *pilD*, *pilF*, *pilT*, and *regG*) are not adjacent to the gene encoding the major pilin subunit (*pilE*), as

TABLE 2. Genetic markers that have been placed on the FA1090 map

Gene designation	Description or phenotype	Reference or source	Zone(s)
adk[a]	Adenylate kinase	E. Feil and B. G. Spratt	E
aniA	Anaerobically induced protein Pan1	16	H
argF	Ornithine transcarbamoylase	11, 23	H
argJ	Ornithine acetyltransferase	11, 24	H
asd	Aspartate semialdehyde dehydrogenase (*N. meningitidis*)	15	B
carB	Carbamoylphosphate synthetase	29	C
cnp	Cryptic *Neisseria* protein (2 adjacent copies in MS11)	11, 32	D
dcmA	M.NgoPI methylase	11, 38	B
dcmB	M.NgoPII methylase	11, 39	A
dcmD	M.R.NgoMI methylase and restriction enzyme	11, 13	F
dcmE	M.NgoBIII methylase	11, 13	F
dcmG	M.NgoDI methylase	11, 13	E
dhp	Dihydropteroate synthase (*N. meningitidis*)	11, 21	I
fbp	37-kDa iron-repressible outer membrane protein (OMP)	2, 11	D
frpB	70-kDa iron-repressible OMP	M. Beucher and P. F. Sparling	C
fur	Ferric uptake regulator	C. Thomas and P. F. Sparling	A
glnA[a]	Glutamine synthetase	H. Seifert; 11	J
gyrA[a]	DNA gyrase subunit	R. Belland	F
gyrB	DNA gyrase subunit	11, 35	A
himA[a]	Integration host factor subunit	R. Belland	D
himD[a]	Integration host factor subunit	R. Belland	E
hsp-63	63-kDa stress protein GSP63	Y. Pannekoek	C
iga	Immunoglobulin A protease	11, 30	D
laz	Outer membrane (OM) lipid-modified azurin with H.8 epitope	11, 44	G
lbpA	Lactoferrin-binding protein	G. D. Biswas and P. F. Sparling	D
lepA[b]	GTP membrane-binding protein (function unknown)	R. Belland	E
lip	OM lipoprotein with H.8 epitope	11, 45	G
lpd[b]	Lipoamide dehydrogenase	R. Belland and S. Porcella	G
lps-1	Lipooligosaccharide antigen	11, 28	D
mtrR	Increased resistance to antibiotics and hydrophobic compounds	W. Pan and B. G. Spratt	I
omc	OMP-macromolecule complex	11, 42	D
opaA	Opacity (Opa) OMP	7, 11	C
opaB	Opacity OMP	7, 11	C
opaC	Opacity OMP	8, 11	G
opaD	Opacity OMP	8, 11	J
opaE	Opacity OMP	8, 11	B
opaF	Opacity OMP	8, 11	G
opaG	Opacity OMP	8, 11	C
opaH	Opacity OMP	8, 11	J
opaI	Opacity OMP	8, 11	I
opaJ	Opacity OMP	8, 11	G
opaK	Opacity OMP	8, 11	H
oxiA	Anaerobically repressed OMP	10, 11	E
parC[a]	Topoisomerase activity	R. Belland	H
penA	Penicillin-binding protein 2	11, 34	J
pilC	Pilus assembly (2 chromosomal copies)	11, 18	B, C
pilD	Prepilin leader peptidase	22	A
pilE	Pilin expression locus	1, 11	C
pilF	Pilin assembly	22	A
pilS	Pilin storage locus (5 chromosomal copies)	11, 14	J, B, C
pilT	Pilus-associated twitching motility and colony morphology	22	B
por	OM porin	5, 11	A
proAB	Proline biosynthesis	11, 36	F
recA	General recombination and DNA repair	11, 20	F
regF	Regulation of *pilE* transcription	H. De Reuse and C. Marchal	C
rmp	Reduction-modifiable OMP	11, 12	J
rrnA	rRNA	37	B
rrnB	rRNA	37	A
rrnC	rRNA	37	H
rrnD	rRNA	37	H
sac-4	Serum resistance	11, 25	A
sucA[a]	α-Ketoglutarate dehydrogenase (decarboxylase)	R. Belland and S. Porcella	G
tbpA	Transferrin-binding protein TBP-1	9, 11	J
tufA[a]	Elongation factor (EF-Tu)	R. Belland and S. Porcella	A
tyrS[a]	Tyrosyl-tRNA synthetase	B. Spratt; 11	C
uvrA[a]	Excision nuclease [DNA repair])	J. K. Davies	H
uvrB[a]	Excision nuclease [DNA repair])	J. K. Davies	E
uvrC[a]	Excision nuclease [DNA repair])	R. Belland	E

[a] The gene designation is based on sequence similarity to previously described genes from *E. coli* or other bacterial species, as determined by the investigator(s) providing this probe; functional characterization of the gene product has not necessarily been done.

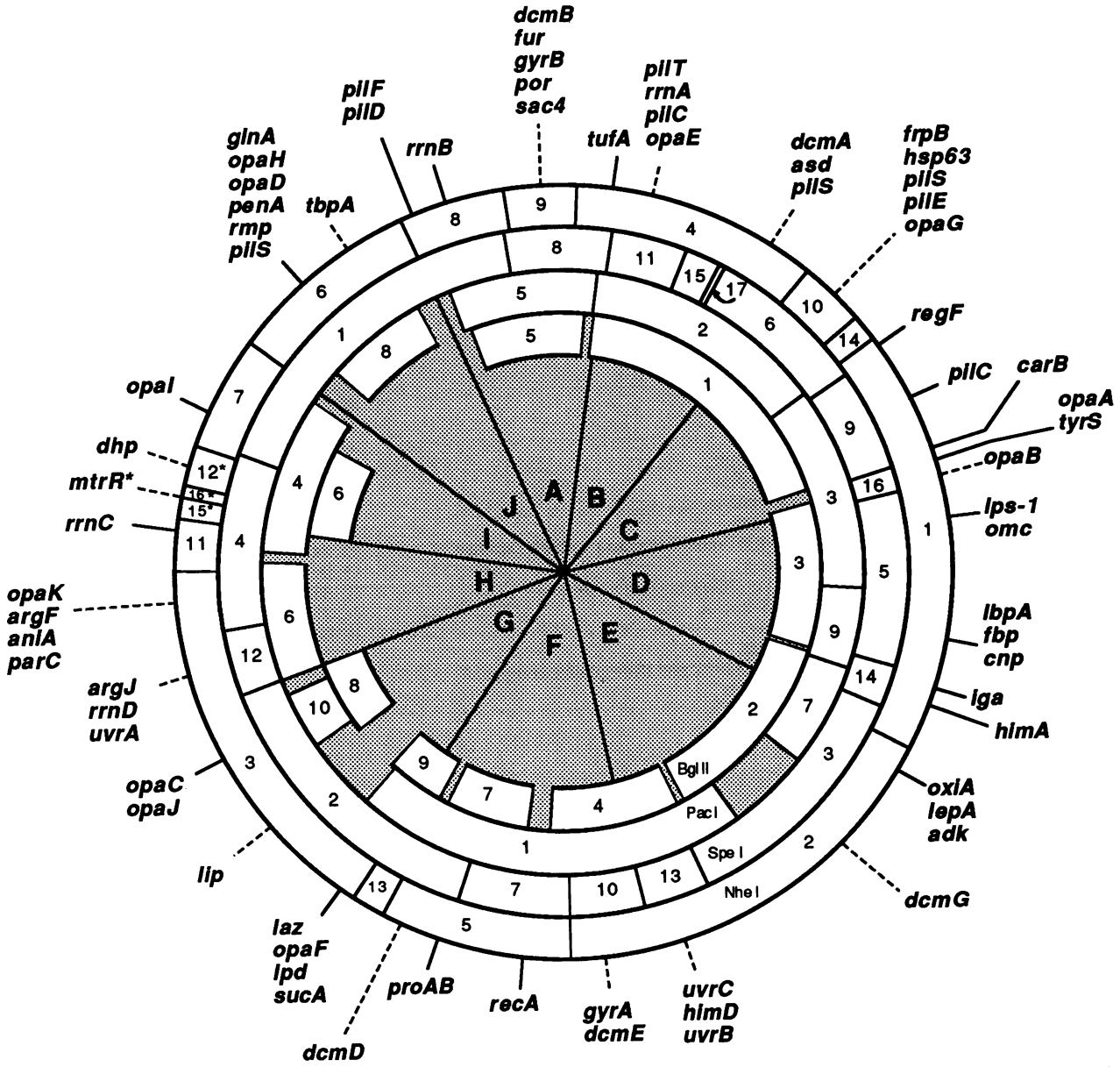

FIG. 1. Macrorestriction map of the strain FA1090 chromosome. A group of markers that map to the same region of fragment overlap is connected to the map by a solid or broken line. Broken lines indicate that markers may be located anywhere within the designated region of overlap between an *Nhe*I fragment and an *Spe*I fragment. Solid lines indicate groups of markers whose positions within *Spe*I-*Nhe*I overlaps were further localized by the pattern of hybridization to *Bgl*II or *Pac*I fragments. The order of markers within each group could not be determined. The three *Nhe*I fragments whose relative order is not known are indicated by asterisks, as is the position of *mtrR*, which could be on either fragment N15 (*Nhe*I fragment 15) or fragment N16. The relative order of fragments N10 and N14, which was not known at the time of publication of the initial macrorestriction map (11), was determined by use of a *pilS* gene clone that contains an *Nhe*I site and is a linking clone for fragments N10 and N4 (33). The positions of fragments P7, P9, P3, and B3 were shifted slightly relative to their original assignments in the *Spe*I and *Nhe*I maps (11), on the basis of the hybridization results obtained with the probes for the *himA*, *regF*, and *carB* genes. The map is divided into 10 arbitrary zones, designated A through J, which are indicated in the center of the map; the zone in which each genetic marker is located is listed in Table 2.

occurs in some other bacterial species (17, 26). Similarly, the genes encoding iron-repressible proteins (*tbpA*, *fur*, *frpB*, *fbp*, and *lbpA*) form a coordinately regulated group, and most of these genes are located in different regions of the chromosome. As additional markers are added to the map and its resolution improves, more insights into chromosome organization and possible mechanisms of gene regulation in the gonococcus will emerge.

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